

Newborn Screening for Pompe Disease and other Lysosomal Storage Disorders: *CDC Reference Methods and Materials*

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Newborn Screening Translational Research Initiative (NSTRI)

Newborn Screening and Molecular Biology Branch

***Technical Workshop on Methods to Detect Pompe Disease and other
Lysosomal Storage Disorders by Newborn Bloodspot Screening***

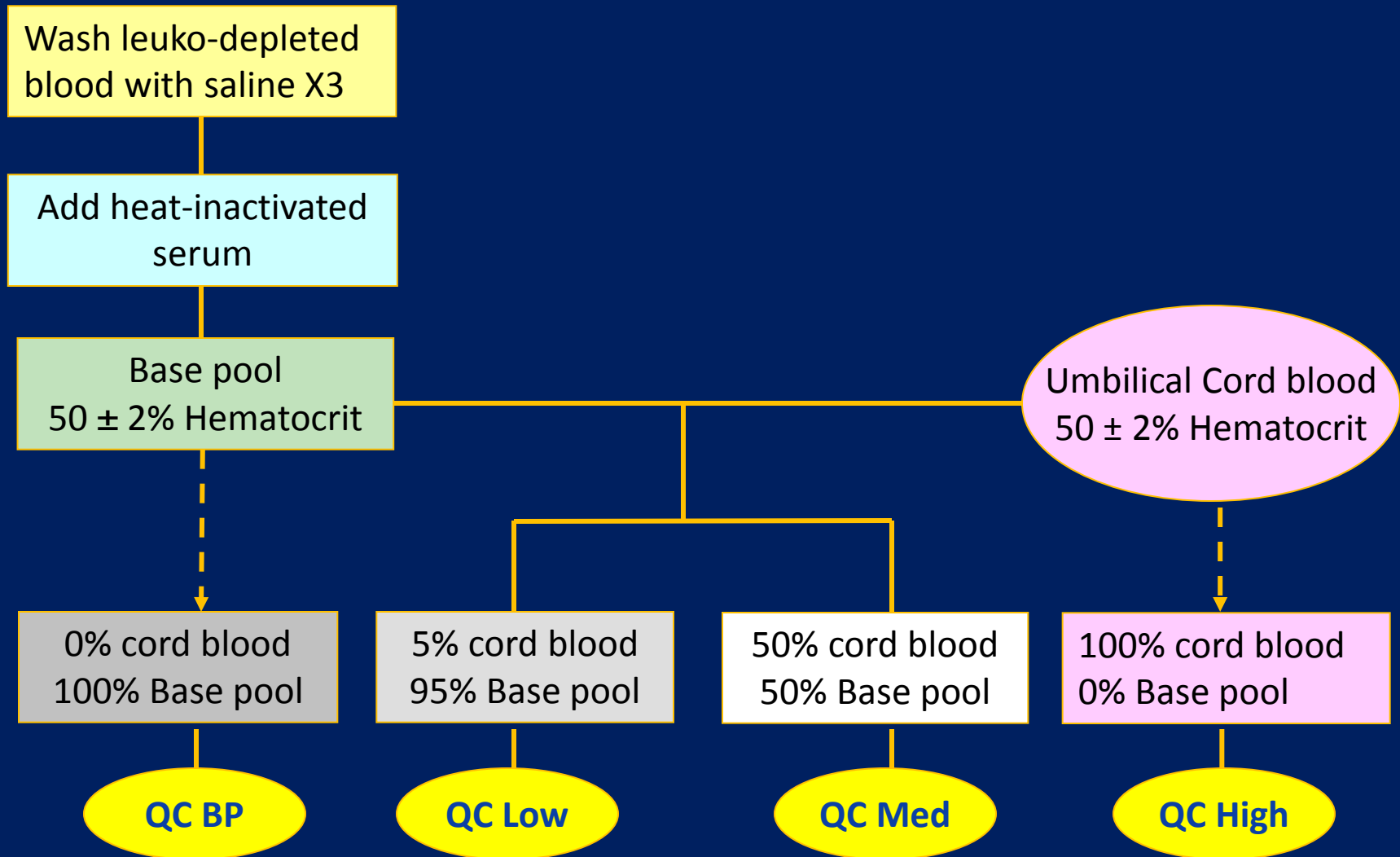
Atlanta, April 16-17, 2015

Disclosures

Dr. Zhou is an NSTRI Senior Scientist who is employed by the CDC Foundation. Her position is supported by donations from Genzyme Corporation.

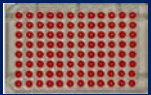
The CDC Foundation receives donations on behalf of NSTRI from Genzyme Corporation, a Sanofi Company; Biogen-Idec; and The Legacy of Angels Foundation.

QC Blood Pool Production



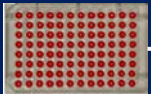
LSD Enzyme Function Assays by FIA-MS/MS

One 3.2mm Punch



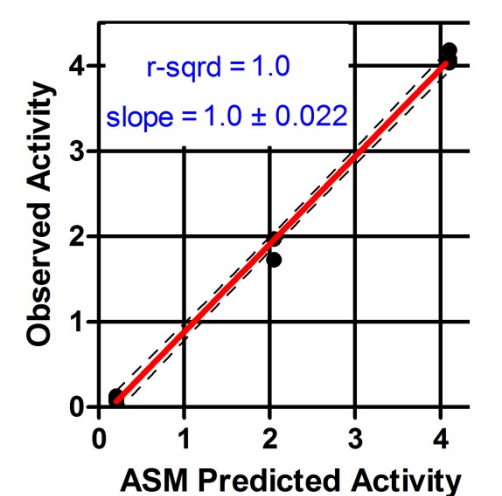
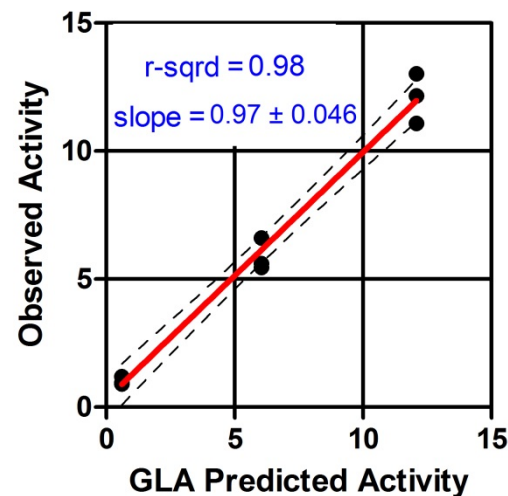
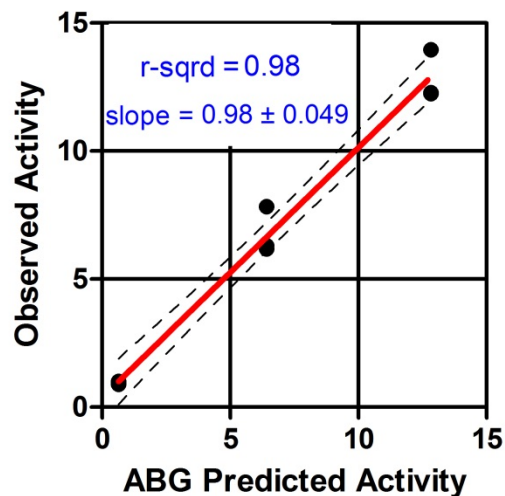
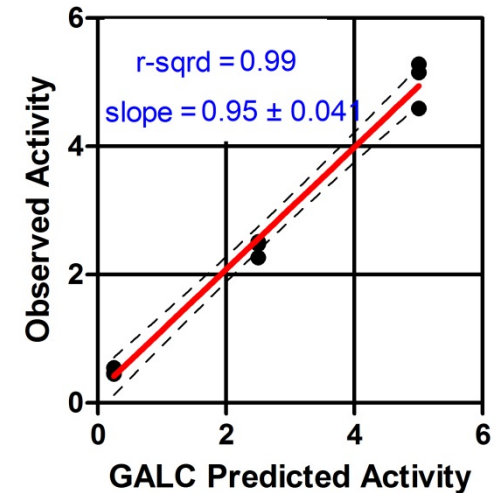
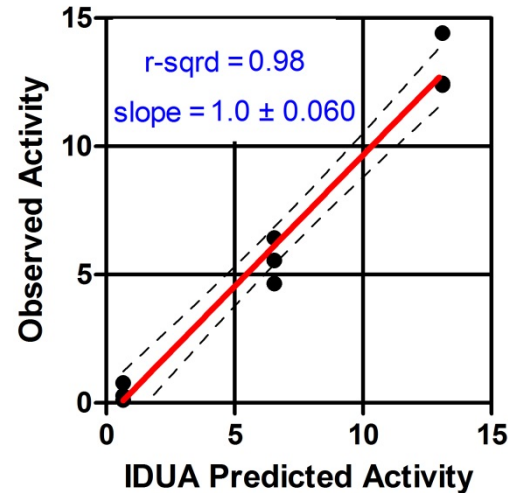
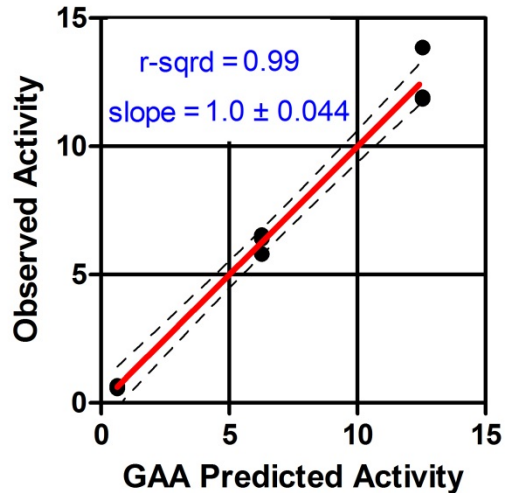
Elute in 70 μ l
37C, 1 hr

One 3.2mm Punch

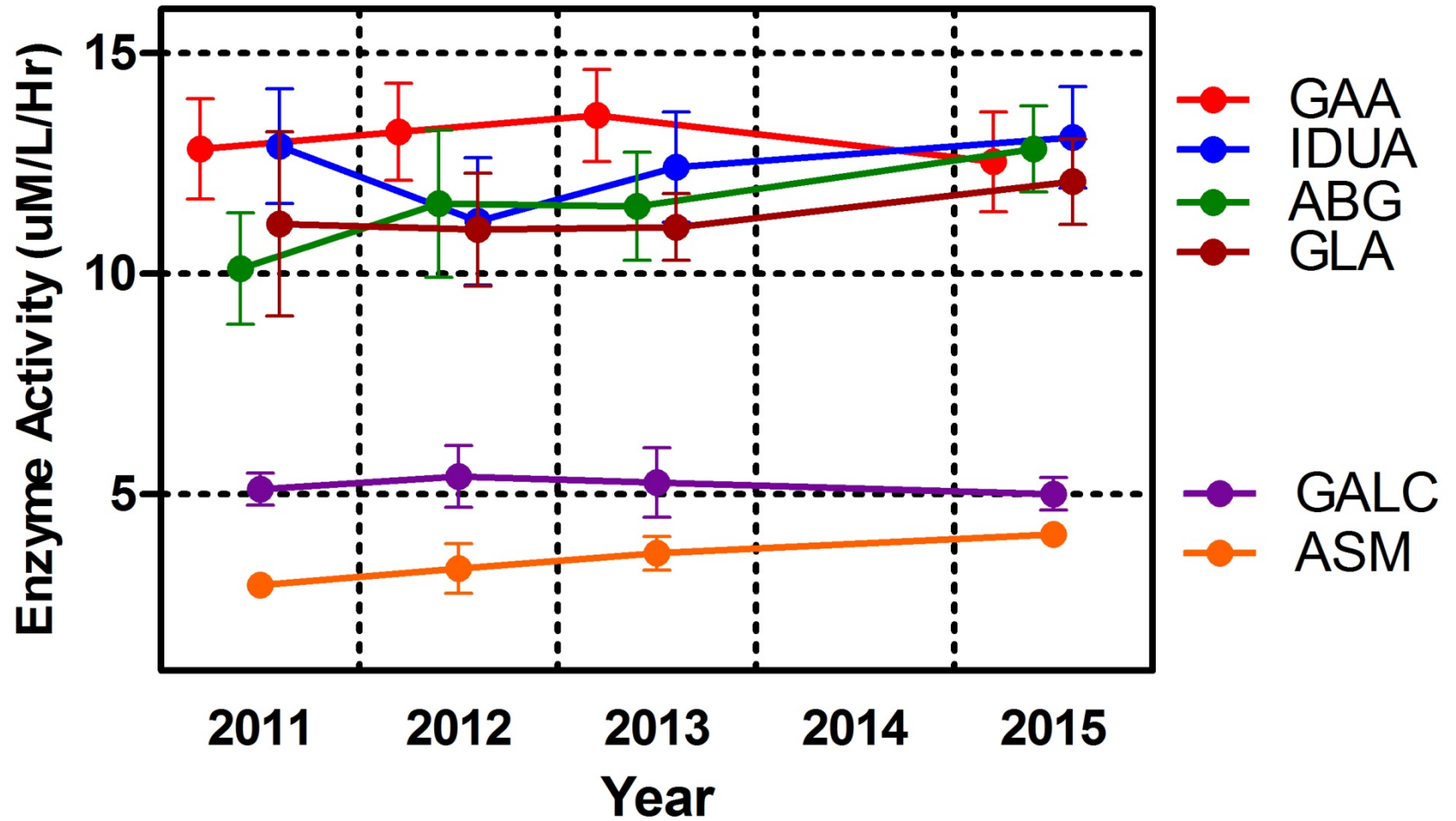


30 μ l GALC
Substrate
Cocktail

Linearity of CDC LSD Reference System

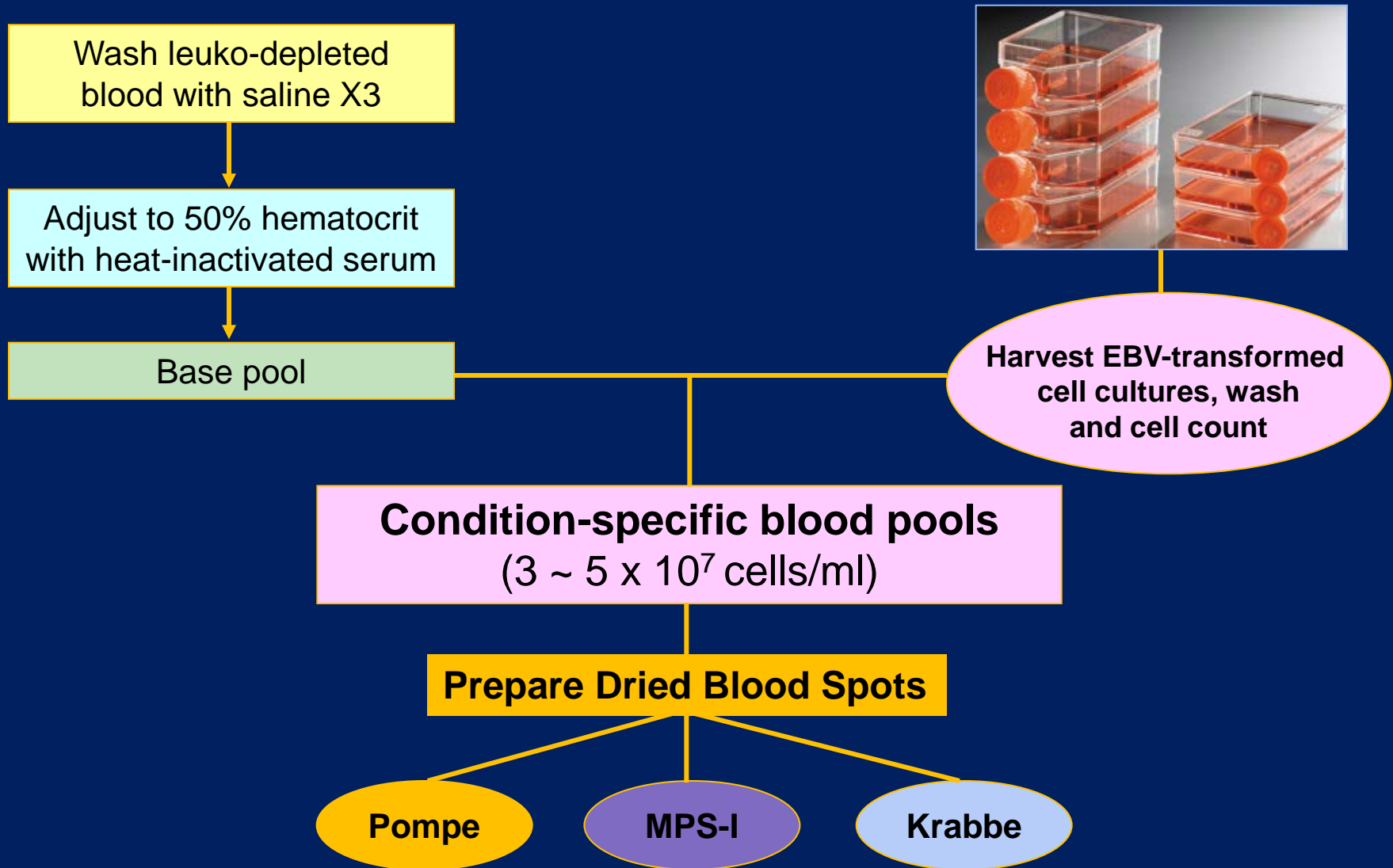


QC-High DBS-RM Over 4 Years

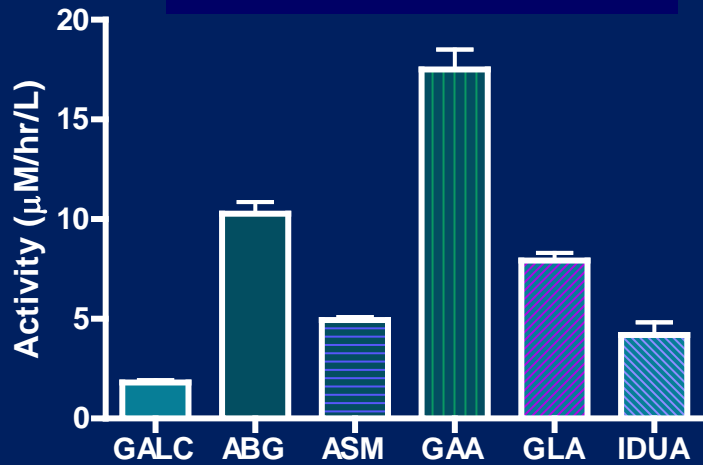


Preparation of Dried Blood Spot Proficiency Testing Materials for Lysosomal Storage Disorders

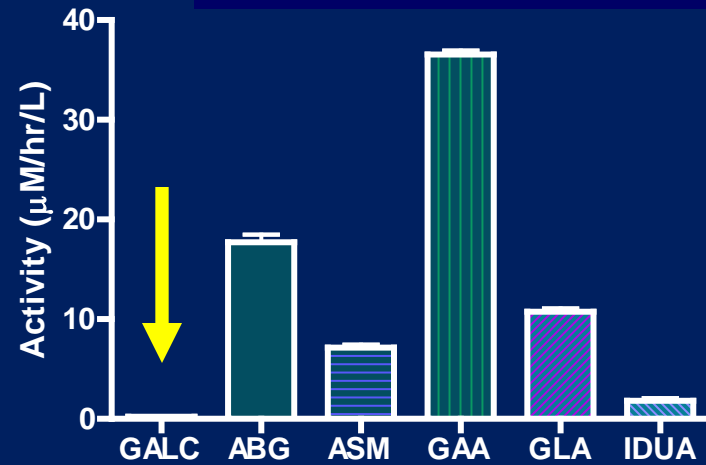
Preparation of Dried Blood Spot Proficiency Testing Materials for Lysosomal Storage Disorders



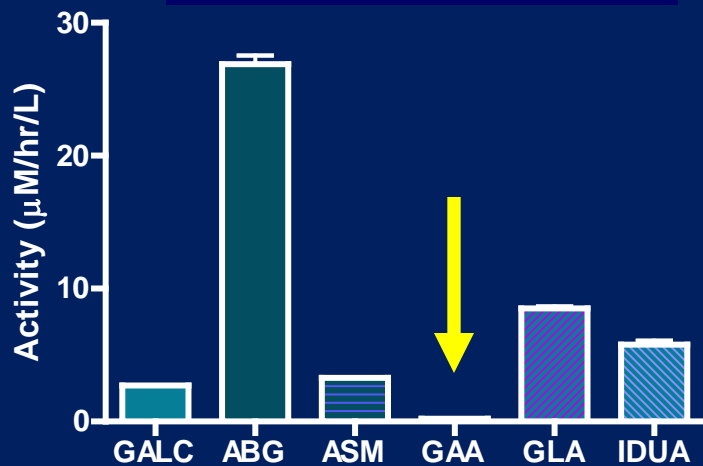
Normal Control DBS



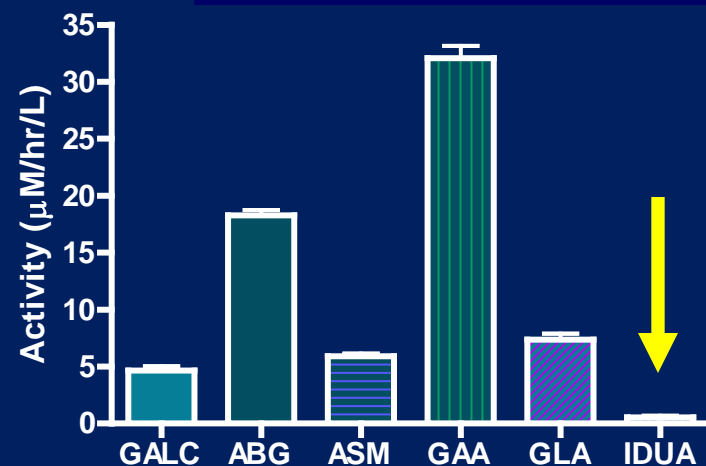
Krabbe-specific DBS



Pompe-specific DBS



MPS I-specific DBS



Inter-Laboratory Evaluation of Condition-specific DBS Reference Material *June 2012*

Condition	# Labs	Normal	Pompe	Krabbe
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Condition-specific DBS are suitable PT material

NSQAP Pilot Proficiency Testing for LSDs

- ❑ In 2013 NSQAP started a pilot proficiency testing (PT) program to detect enzyme deficiencies in dried blood spots for two Lysosomal Storage Disorders
 - Pompe Disease
 - Krabbe Disease
- ❑ US laboratories performing / establishing LSD NBS tests were invited to participate
- ❑ MPS-I will be included in the last send-out of 2015

LSD-NBS PT Sendouts

- ❑ DBS with “normal” enzyme activity were prepared from freshly-collected umbilical cord blood
- ❑ Normal and condition-specific DBS were randomized into 5-member PT panels
- ❑ PT panels were sent nine times between January 2013 and January 2015

Proficiency Testing Performance Metrics for Pompe and Krabbe in DBS*

Condition	No. Labs Reporting	Positive Specimens Assayed (N)	False Negative Errors (%)	Negative Specimens Assayed (N)	False Positive Errors (%)
Pompe (GAA)	7	53	0	212	0.94
Krabbe (GALC)	6	48	0	192	0

*Data compiled from 9 quarters, 5 specimens per proficiency testing panel
January 2013- January 2015

Analytical Summary Data for GAA Activity in Proficiency Testing* DBS (Pompe)

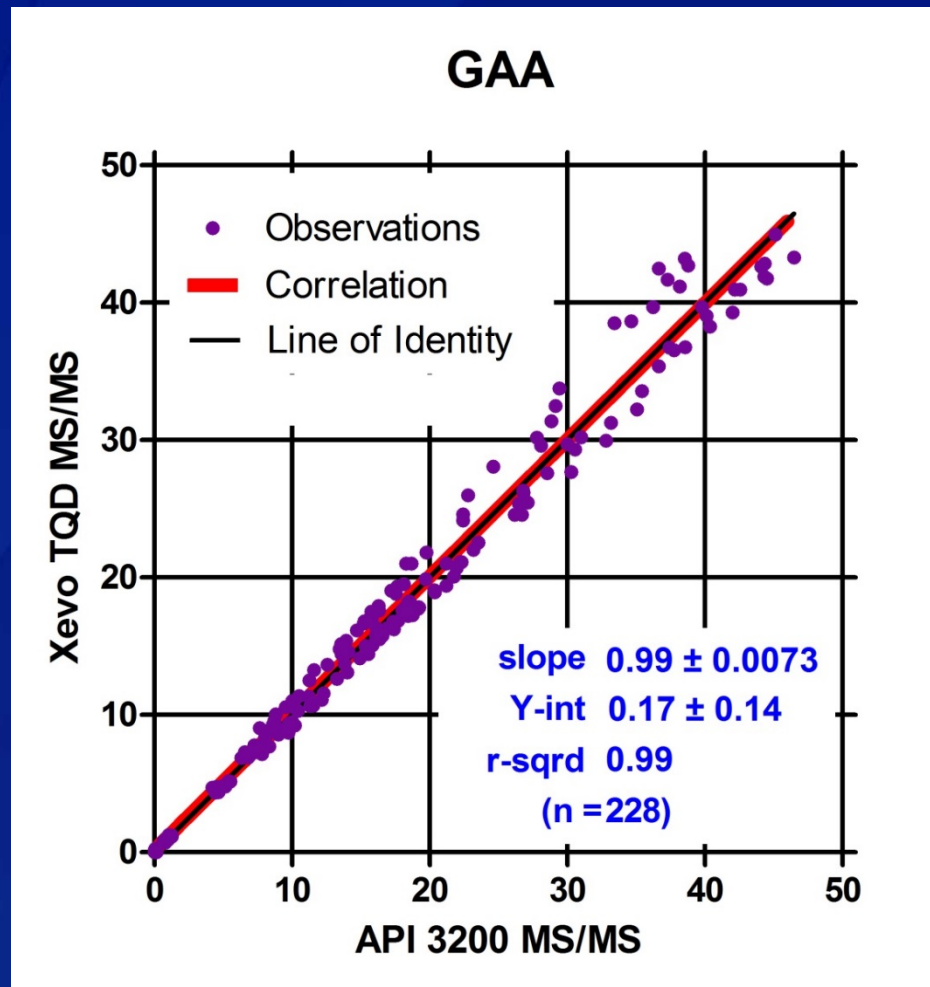
Expected Results (GAA Activity μmol/L/hr)	Normal-1		Normal-2		Normal-3		Pompe Abnormal		Cutoff		
	19.3		17.2		24.8		0.2				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	Min	Max
Flow Injection Analysis MS/MS Non-Kit (N=28)	18.3	4.2	14.9	4.0	21.4	5.1	0.4	0.4	2.9	1.5	4.0
LC-MS/MS (N=5)	3.5	0.4	2.3	0.2	3.9	0.6	0.9	0.1	1.5	1.0	2.9
Digital Microfluidics (N=6)	31.6	2.7	23.5	1.1	36.0	3.4	2.8	0.4	7.2	7.0	8.0

*Six quarters 2013-2014.

Evaluation of an Alternative LSD-NBS MS/MS Test System in Development

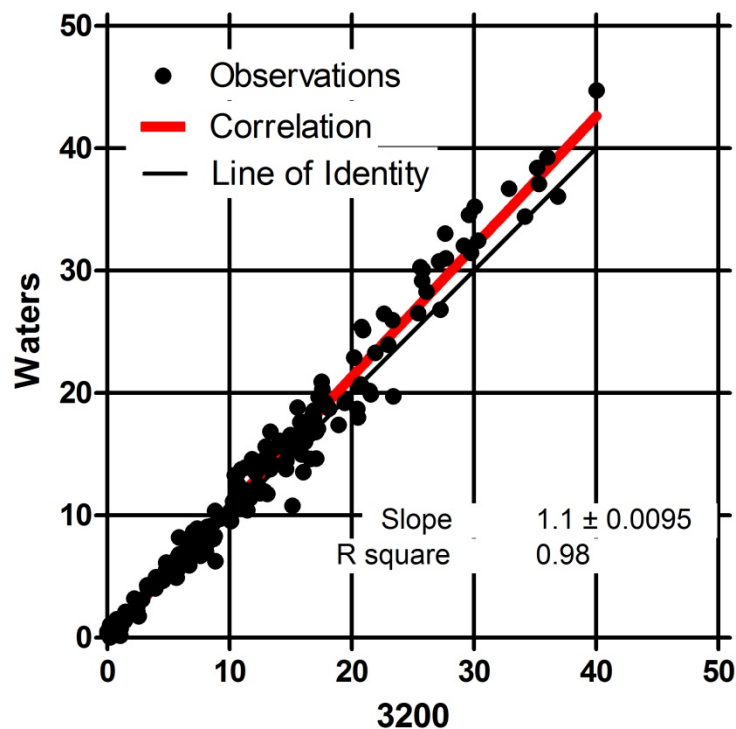
**Intra-Lab Evaluation
(Instrumentation & Reagents)**

Correlation of Enzyme Activity Measured on Two Different MS/MS Instruments

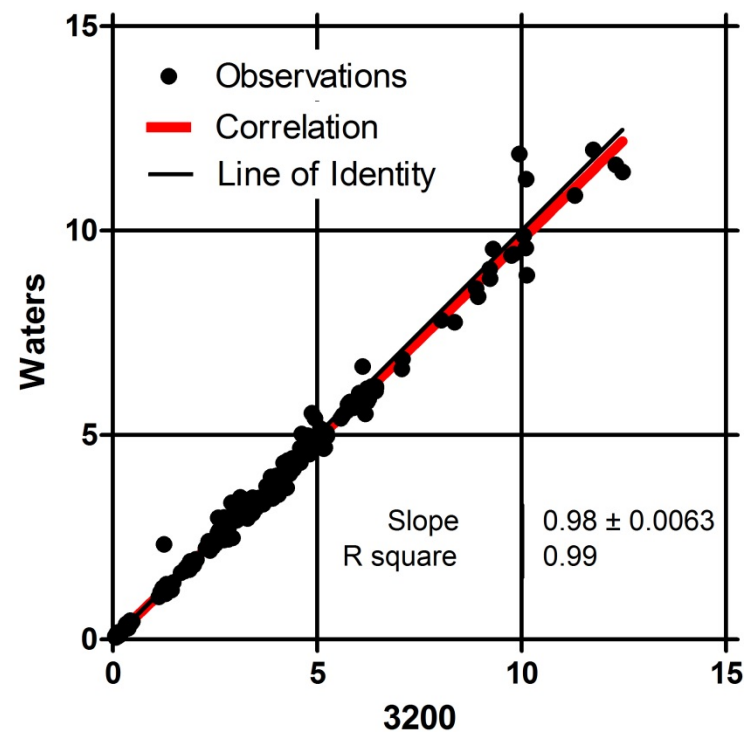


Correlation of Enzyme Activity Measured on Two Different MS/MS Instruments

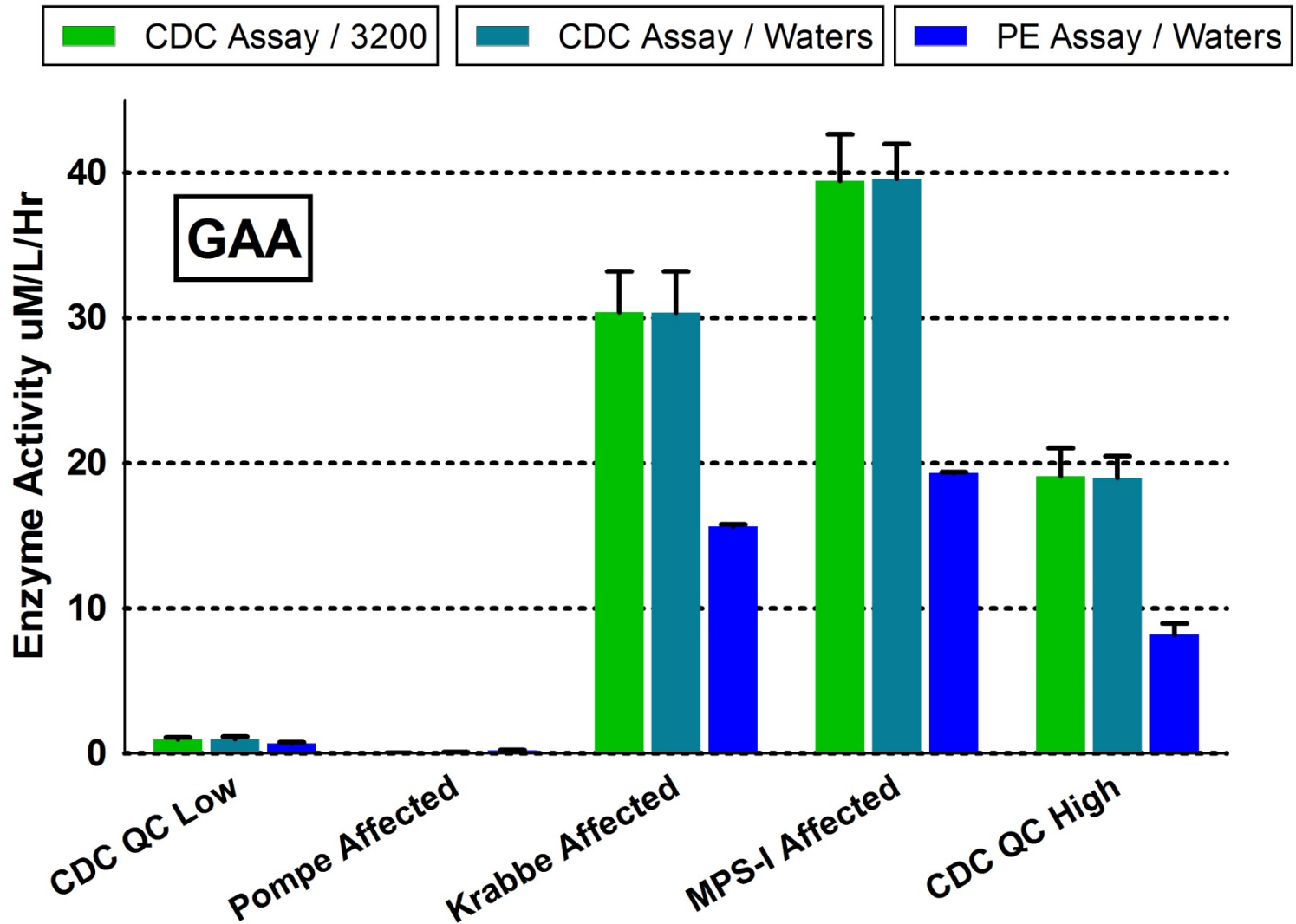
IDUA



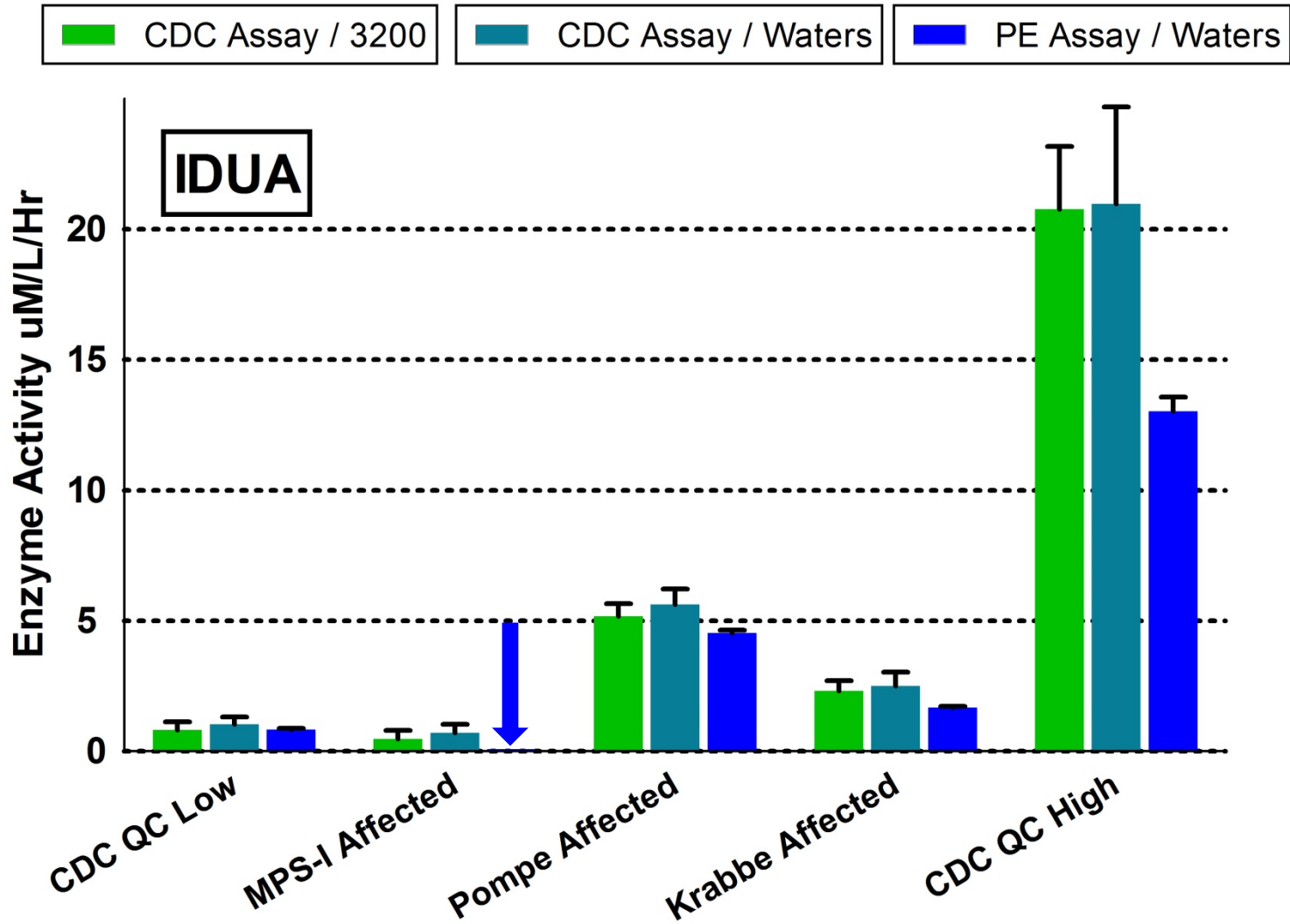
GALC



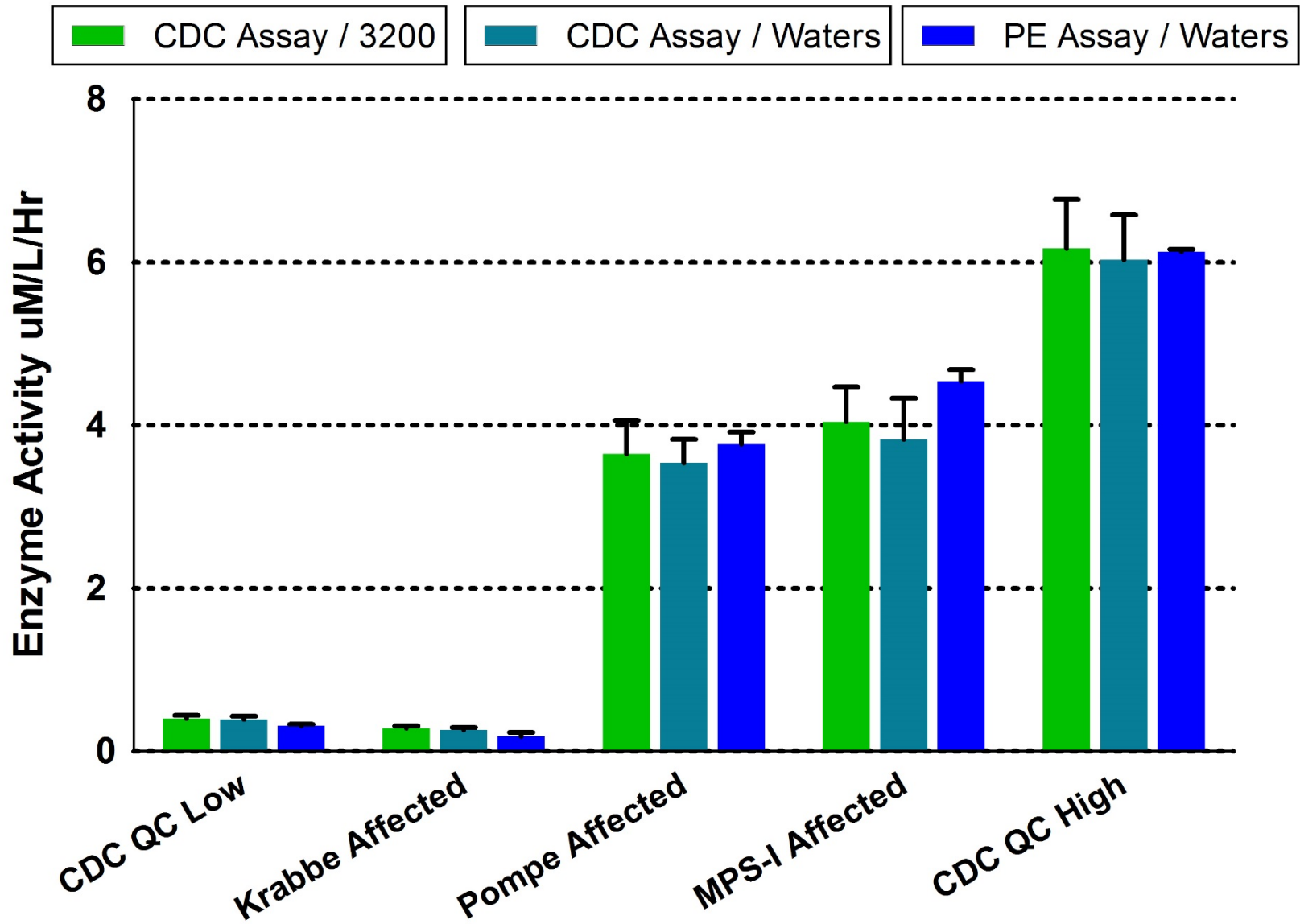
CDC Lab Results in Condition-Specific DBS-RM



CDC Lab Results in Condition-Specific DBS-RM



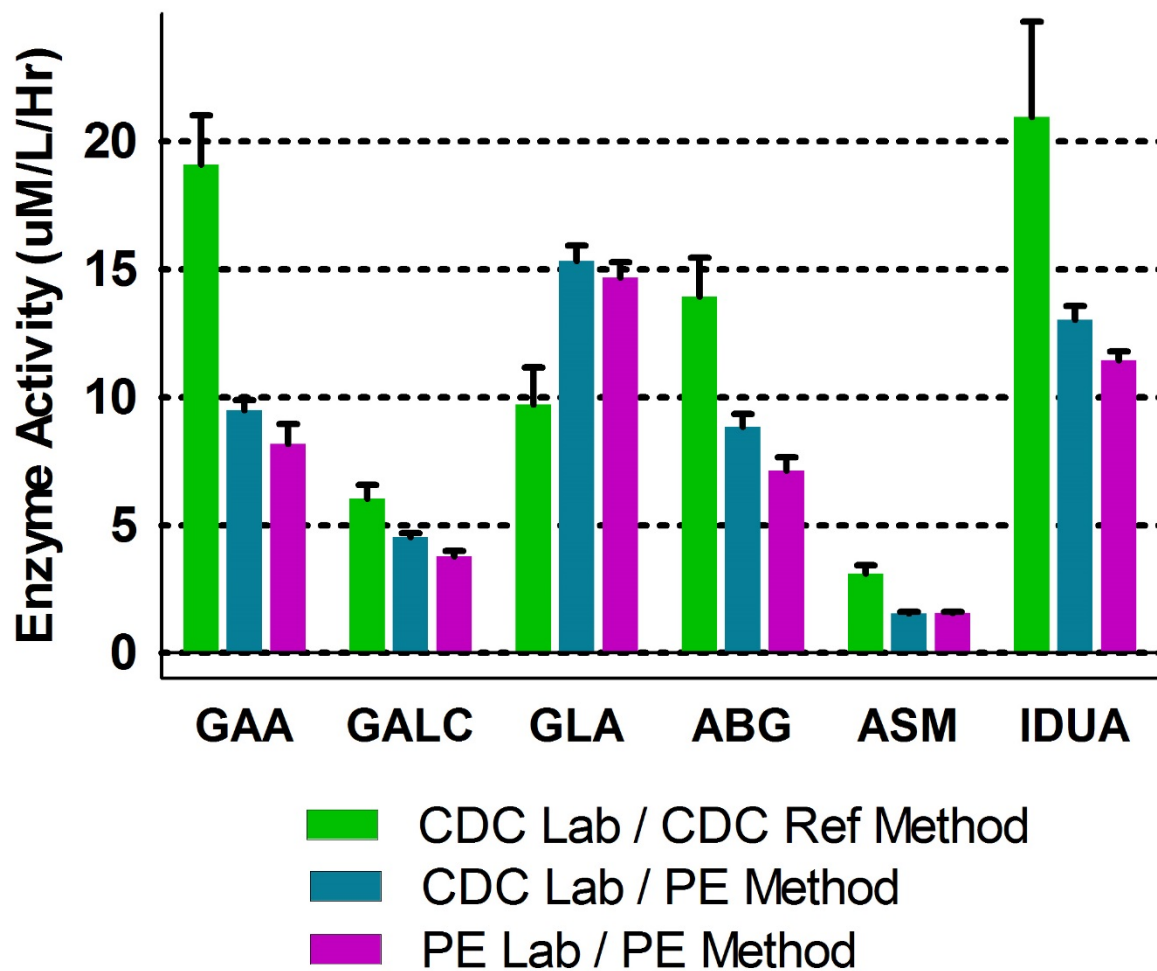
CDC Laboratory Results for GALC



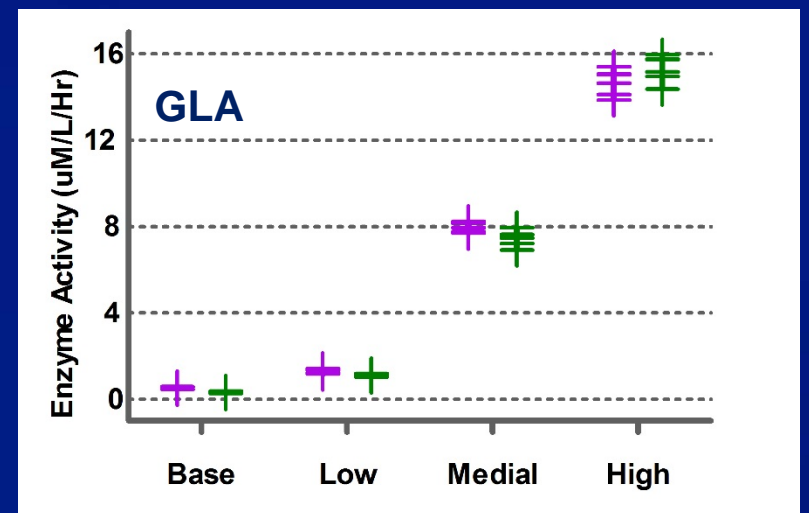
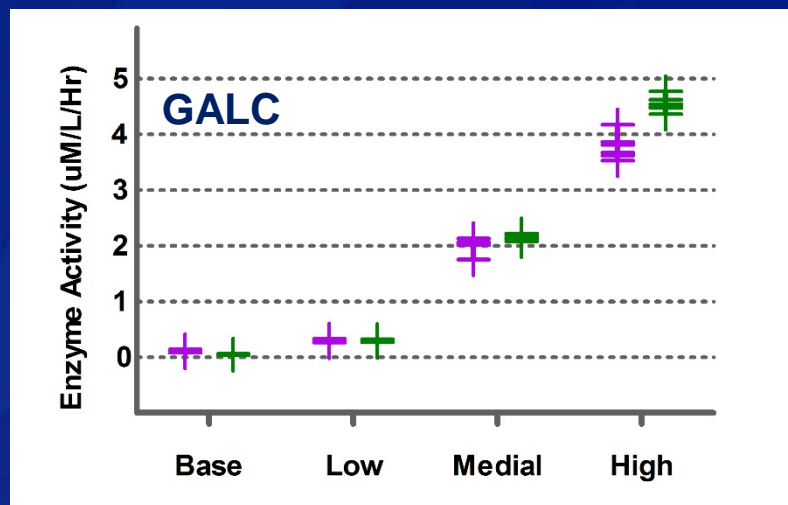
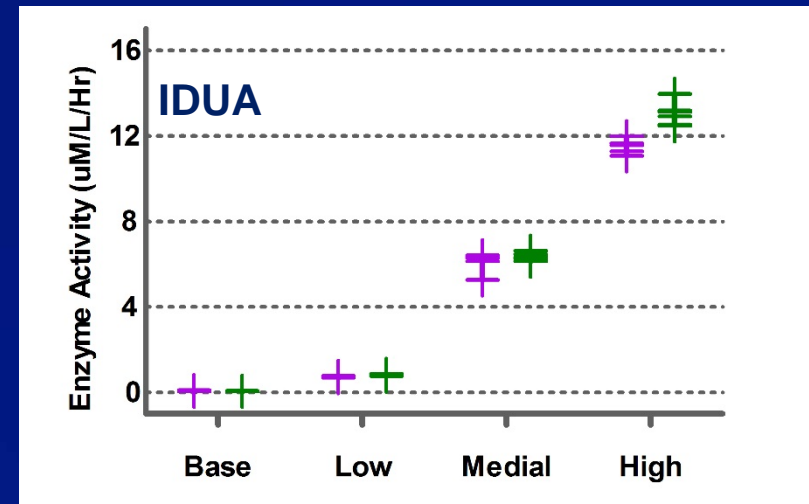
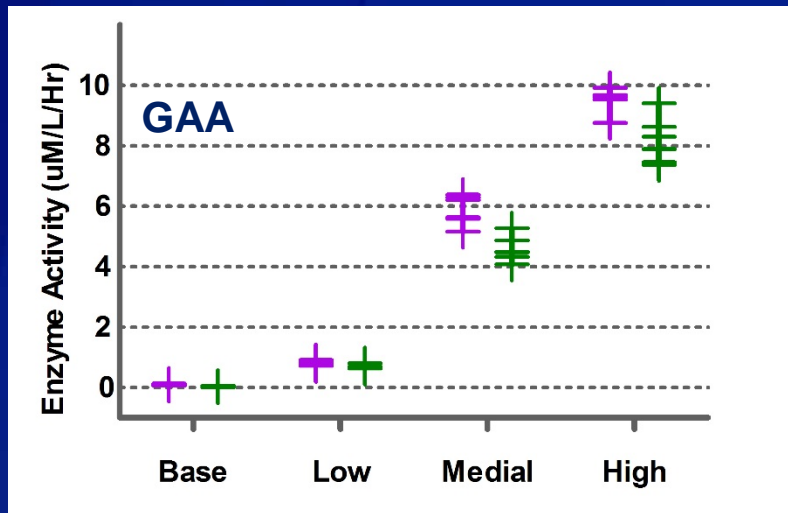
Evaluation of an Alternative LSD-NBS MS/MS Test System in Development

Inter-Lab Evaluation

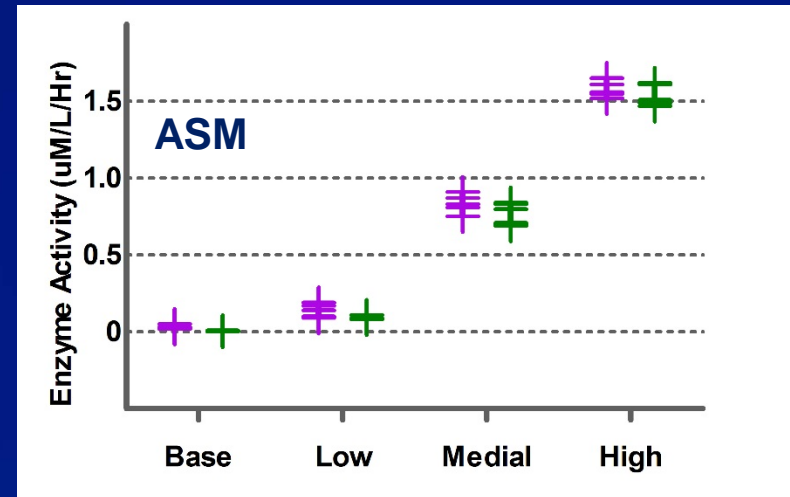
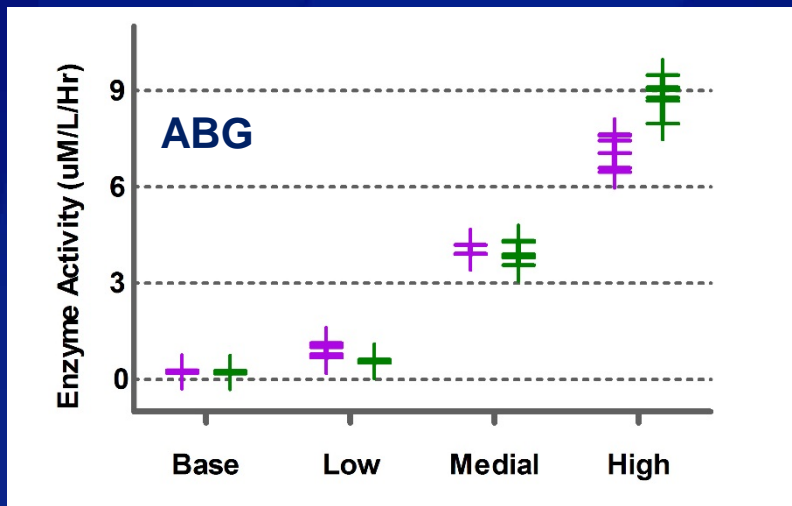
Interlaboratory Results for DBS QC High



Inter-laboratory Evaluation of QC DBS by Fully-Multiplexed FIA-MS/MS



Inter-laboratory Evaluation of QC DBS by Fully-Multiplexed FIA-MS/MS





Thank you for your attention!

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